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DOI:
10.1016/j.watres.2016.07.019

Document Version
Peer reviewed version

Citation for published version (Harvard):
Jones, A & Bridgeman, J 2016, 'Investigating the characteristic strength of flocs formed from crude and purified Hibiscus extracts in water treatment', *Water Research*, vol. 103, pp. 21-29.
https://doi.org/10.1016/j.watres.2016.07.019

Investigating the characteristic strength of flocs formed from crude and purified Hibiscus extracts in water treatment

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Abstract

The growth, breakage and re-growth of flocs formed using crude and purified seed extracts of Okra (OK), Sabdariffa (SB) and Kenaf (KE) as coagulants and coagulant aids was assessed. The results showed floc size increased from 300µm when aluminium sulphate (AS) was used as a coagulant to between 696µm and 722µm with the addition of 50mg/l of OK, KE and SB crude samples as coagulant aids. Similarly, an increase in floc size was observed when each of the purified proteins was used as coagulant aid at doses of between 0.123 and 0.74mg/l. The largest floc sizes of 741µm, 460µm and 571µm were obtained with a 0.123mg/l dose of purified Okra protein (POP), purified Sabdariffa (PSP) and purified Kenaf (PKP) respectively. Further coagulant aid addition from 0.123 to 0.74mg/l resulted in a decrease in floc size and strength in POP and PSP. However, an increase in floc strength and reduced d_{50} size was observed in PKP at a dose of 0.74mg/l. Flocs produced when using purified and

crude extract samples as coagulant aids exhibited high recovery factors and strength. However, flocs exhibited greater recovery post-breakage when the extracts were used as a primary coagulant. It was observed that the combination of purified proteins and AS improved floc size, strength and recovery factors. Therefore, the applications of Hibiscus seeds in either crude or purified form increases floc growth, strength, recoverability and can also reduce the cost associated with the import of AS in developing countries.

Keywords: Hibiscus extracts, floc strength, coagulants, purified proteins, water treatment

1.0 Introduction

For decades, different chemicals have been applied in water treatment to aid the removal of contaminants and harmful substances. Chemical coagulants are added to destabilise the dispersed colloids, with charge neutralisation, adsorption and sweep flocculation being the major mechanisms of action (Duan and Gregory, 2003). Accelerated sedimentation is achieved by aggregating the flocs via slow mixing (flocculation) to form larger macro flocs facilitating removal in a sedimentation tank. However, to achieve satisfactory treatment, flocs must demonstrate sufficient strength so as not to be broken by the turbulent flow field found in the flocculator and clarifier. Thus, the merit of each coagulant is judged based on, *inter alia*, the strength, size and density of the flocs formed. Previous work has observed that smaller flocs are more likely to resist rupture than larger flocs but may pose some challenges during removal compared to bigger flocs (Boller and Blaser, 1998, Jarvis et al., 2005c), as the mechanism and general mode of floc transportation is hampered if the flocs are small in size and so cannot settle effectively. Conversely, it can be argued that smaller and more compact flocs with tighter bonds will resist breakage and settle faster than larger, weaker flocs (Jarvis et al., 2005c). However, it has been reported that the stronger the flocs, the larger they can

grow under certain shear conditions (Mühle, 1993). However, (Sharp et al., 2006a) revealed that larger flocs can easily break in high turbulent condition, because they are weaker. It can be deduced here that highly compact flocs are generally stronger and smaller in size. Thus, it is challenging to prevent floc breakage under normal plant conditions, particularly in highly turbulent areas; consequently, the regrowth potential of flocs post-rupture is of interest.

Many researchers have investigated floc properties, including floc strength, using different coagulants and under different plant operating conditions. Previous work has monitored floc growth, breakage and re-growth phases after the introduction of high shear rate (Jarvis et al., 2005b, Yu et al., 2012, Xu et al., 2014). Yukselen and Gregory (2004) and Li et al. (2007) observed in their separate studies that AS flocs exhibit irreversible breakage. Conversely, Yu et al. (2014) evaluated the property of kaolin-alum flocs at low pH and showed that 100% floc recovery is possible if AS or Kegging polymer Al13 $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$ was used as coagulant at acidic pH. Several others workers have also reported the importance of low pH in improving floc strength and recoverability using different chemical coagulants (Cao et al., 2010, Sun et al., 2011). Sharp et al. (2006b) investigated the properties of ferric-NOM flocs and revealed that flocs generated by iron salts are larger and more resistant to breakage than AS flocs, resulting in accelerated settling. Beside AS producing irreversible, smaller and weaker flocs (Yukselen and Gregory, 2004, Sharp et al., 2006b, Li et al., 2007), research has also been undertaken to investigate the relationship between residual aluminium in water and Alzheimers disease (Gauthier et al., 2000, Flaten, 2001). Cost issues associated with the import of coagulants such as AS exacerbate these issues further for developing countries. It is, therefore, imperative to search for alternative natural coagulants and coagulant aids that will lower the cost of water treatment in developing countries and also improve water treatment efficiency. By so doing, the number of deaths resulting from

drinking contaminated water supply could be lowered in rural areas and life expectancy increased.

Recently, several natural materials have been studied to assess their coagulation potential in water treatment. Preliminary investigation of some of these natural extracts has so far provided encouraging results for people in developing countries. Naturally-occurring plant extracts including *Moringa oleifera* (MO), *Cactus latifaria*, and *Mustard seeds*, have coagulation capability and can be used in water treatment (Jahn Samia, 1998, Diaz, 1999, Bodlund et al., 2014). Similarly, other natural plants, such as Hibiscus, are widely used in many tropical countries because of their nutritional values. Among the many Hibiscus plant species, only OK seed pod has been investigated as a flocculant in the treatment of water and wastewater (Agarwal et al., 2001, de Jesus et al., 2013). Recently, Jones and Bridgeman (2016) have demostrated the capability of OK seed extract in removing turbidity and bacteria in river water. Additionally, it has been reported that activated carbon derived from KE fibre, another Hibiscus plant could be used to treat water and wastewater with high heavy metal contents (Chowdhury et al., 2012). Conversely, there is no known report on the use of SB seed in either water or wastewater treatment. However, SB extract was found as an effective inhibitor of microbial growth when it was applied on some isolated microbes (Nwaiwu et al., 2012). Most of the reported work has centred on the coagulation activities of the extracts, whereas problems related to floc strength and recovery have not been investigated, despite their importance in the treatment process. Therefore, the aim of this study was to investigate the potential of using Hibiscus plant as a primary coagulant and as a coagulant aid, and to assess the floc characteristics in terms of floc size, strength, and recovery ability.

2.0 Materials and methods

2.1 Collection and preparation of the seeds

All the seeds used in this study, OK, KE and SB, were obtained from a local market in Nigeria. The seeds were manually prepared by removing the seeds from the capsules and pods to access the seed kernels. The seeds were cleaned by washing with tap water to remove contaminants such as stones, plant debris and dust and then dried in an oven at 60ºC for six hours. The dried seeds were ground into a fine powder for 2 minutes using a Tema laboratory disc mill. The ground seed powders were then sieved and the powder retained in the 212 µm, and 300 µm sieve sizes was combined and subsequently used in the preparation of the coagulants.

2.2 Chemicals and reagents

Analytical grade sodium chloride, aluminium sulphate and hydrochloric acid (Fisher Scientific, UK), kaolin Fluka-60609, (Sigma-Aldrich, Germany), sodium phosphate monobasic monohydrate (Sigma-Aldrich, Germany), and sodium phosphate dibasic (Sigma-Aldrich, UK) were used in the study. Deionized (DI) water was used to prepare all suspensions and concentration solutions.

2.3 Preparation and extraction of the natural seed coagulants

1M sodium chloride (NaCl) solution was prepared by dissolving 58.5 g NaCl in 1000 ml of DI water to obtain the required concentration. The crude seed extract (CSEs) were prepared from the ground seed powders by adding 1.0 M NaCl solutions to the seed powder to make 2% (w/v) suspension. The suspension was stirred vigorously using a magnetic stirrer for 15min at room temperature (19±2ºC). The suspension was then centrifuged at 4500 rpm for 10 minutes using a Heraeus Megafuge16 (Thermo Scientific, Germany). The suspension was decanted and the residual solids dried in an oven at 50ºC overnight. The weight of the dried solid material was measured to ascertain the amount of seed powder used in making the

suspension. The decanted suspension was then filtered through a Whatman No. 42 filter paper. The filtrates were termed crude extracts and were then used as primary coagulant or coagulant aids in a series of jar test experiments.

2 g of AS powder was dissolved in 100 ml of DI water and the suspension rapidly mixed for 15 minutes, using a magnetic stirrer. This AS coagulant was applied in the jar test experiments to determine the optimum coagulant dose required in the strength test.

2.4 Protein purification and lipid extraction from the seed

The ground seed powders (212µm−300µm) were defatted using high-grade hexane in an electro-thermal Soxhlet extractor. 20g of the seed powder was used during the extraction. For efficient extraction, 2L of solvent volume (hexane) was used and heated to 60 °C. The process was run continually for 8 hours with each complete cycle taking 2 to 3 minutes. The residues were dried overnight at room temperature (19±2°C) and the dried residue was ground into a fine powder using pestle and mortar and was applied in the subsequent purification processes.

2.4.1 Purification by ion exchange column chromatography

A HiTrap Q HP (1 ml) anion column, (GE Healthcare, Sweden) was used for the purification of the protein of interest of the hibiscus plants. The column connected to a pump (Watson-Marlow Breeder pump 323, UK), and the pump head adjusted to a flow rate of 1 ml per minute. The preservatives were washed with 10ml of DI water, followed by ten column volumes (CV) of 1 M NaCl dissolved in the phosphate buffer. The column was then equilibrated with the phosphate buffer 10 CV before loading the protein. 5g of the oil-free powder was dissolved in 0.1 M phosphate buffer and mixed thoroughly for one hour using a

magnetic stirrer. The mixture was centrifuged at 20,000 rpm at 4°C for 40 minutes before decanting the supernatant. The supernatant was injected using a peristaltic pump onto the ion exchange column to separate the protein of interest from the contaminants.

The sample was loaded at a flow rate of 1 ml per minute, where the protein of interest was bound to the Column matrix throughout the loading process. The weakly bound contaminants were washed away with the equilibrating (initial) buffer using 10 CV. The proteins of interest were eluted, beginning with, 0.3, 0.5 and 1.0 M of NaCl–phosphate buffers and the various fractions collected. The collected fractions were analysed for absorbance using a spectrophotometer (Varian Carey 50 probe UV-visible, Australia) and coagulation performance using a standard jar tester (Phipps and Bird, 7790-900B USA). The purified protein contents were evaluated for floc strength using a laser diffraction particle size analyser (Mastersizer, Malvern 2000, UK).

2.5 Preparation of the synthetic turbid water

Turbid water samples for the jar test experiments were prepared by adding kaolin particles to tap water. 40 g of laboratory grade kaolin (Fluka and high-grade, Sigma-Aldrich) was added to 400ml of tap water, and the suspension stirred for 30min using a magnetic stirrer. The suspension was made up to 1L by adding 600ml of tap water and then stirred for a further 30min. The suspension was allowed to stand for 24hr for the kaolin to hydrate and then allowed to stand for another seven days. The supernatant was decanted, and 0.3ml of the stock solution was mixed with 1L of tap water to produce turbidity value ranges of 46 ± 1NTU.

2.6 Jar test experiments

Jar tests were conducted using a standard apparatus (Phipps and Bird, 7790-900B, USA) comprising six 1L beakers to evaluate the optimum coagulant dose for the coagulation tests.

For effective dispersion of the coagulant, the water was rapidly mixed at 200 rpm for 1.5 minutes during which time various doses of the coagulant were added to the beakers. The mixing speed was then reduced to 30rpm for a further 25 minutes to simulate the flocculation stage. The suspension was then allowed to stand undisturbed for 1 hour to facilitate settlement. The long sedimentation time was adopted in order to assess the effectiveness of the process and to see whether the requirement to filter might be avoided after prolonged settlement for people in rural areas. A final treated water sample (10 ml) was drawn via syringe 2cm from the top surface of the water in the beakers. Both initial and final water turbidity were then measured using a turbidity meter (HI 93703, Hanna). In a separate experiment using river water, residual dissolved organic carbon (DOC) was measured in water before and after treatment with crude and purified samples. DOC measurement was conducted using TOC analyser (Shimadzu TOC-V-CSH). All experiments were performed at room temperature (19 ± 2ºC).

2.7 Floc formation, breakage and reformation experiments

To assess floc regrowth, flocs were broken by introducing rapid mixing at 200 rpm for 1.5 min. The rotor speed was then reduced to 30 rpm for 25 min to determine the floc re-growth capability of the various coagulants. To compare the flocculation capacity of the seed extracts as coagulant aids, a predetermined dose was added before the end of the coagulation test with AS as a primary coagulant (i.e. 45s after the AS was dosed).

Floc growth, breakage and re-growth were assessed using a laser diffraction instrument (Mastersizer 2000, Malvern, UK), following (Jarvis et al., 2005b, Li et al., 2007, Yu et al., 2012). The Mastersizer was connected to a jar test apparatus and the liquid suspension was monitored by continuously drawing water through the optical unit of the Mastersizer and returning to the jar tester, as shown in Fig. 1.

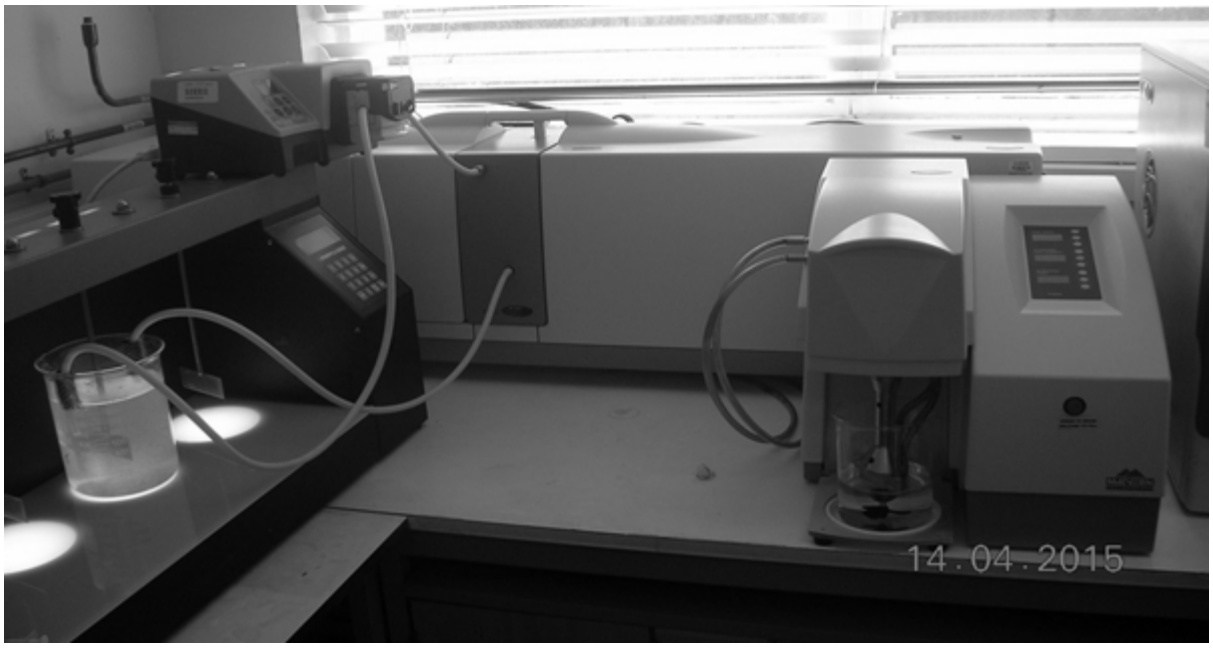


Fig 1 Systematic connection of the Mastersizer 2000 and a jar test apparatus for floc properties monitoring.

Pumping was via a peristaltic pump (Watson-Marlow, 323S, USA) positioned on the return tube with 4.8 mm internal diameter peristaltic pump tubing. The inflow and the outflow were located 10 mm above the blade of the jar tester and opposite each other. Measurements were taken every 35s for the duration of the experiment, and the results automatically logged onto a computer. The flow rate was kept at 2 L/hr (i.e. @33.3 ml/min) throughout the experiment to avoid either floc breakage or floc settling in the tubing. The coagulant dosage was the dose obtained from the earlier jar test results as described above.

2.7.1 Floc strength and floc recovery factors

To understand the properties of the coagulated flocs, it is important to consider the floc strength and floc recovery after exposure to high shear. Floc strength reveals the resistance of the flocs to stress and can be described using a strength factor. Similarly, the recovery factor reveals the ability of a floc to re-grow after breakage. Floc strength and recovery factors were calculated using Equations 1 and 2 following (Sun et al., 2011, Xiao et al., 2011, Yu et al., 2014):

$$S_f = \frac{d2}{d1} \times 100 \quad \text{Eq. 1}$$

$$R_f = \frac{d3-d2}{d1-d2} \times 100 \quad \text{Eq. 2}$$

where d_1 is the average median floc size established at the steady phase before breakage, d_2 is the median floc size achieved after it was subjected to high shear rate. The average median size, d_3, is the average median floc size achieved at the final steady phase after floc breakage.

3.0 Results and discussion

3.1 Floc growth and size of Hibiscus seed extracts as primary coagulants

The results of the floc formation and breakage experiments using crude extracts of OK, SB and KE and AS as primary coagulants are shown in Fig 2. 50 mg/l dose of each extract was employed as primary coagulants in this work, and the median d_{50} floc size was considered throughout the study. The concentration of proteins in the extracts was 1.018 mg/ml in OK, 0.918 mg/ml in SB and 0.631 in KE respectively. Fig 2 shows that the median floc sizes for SB and KE were approximately 176µm and 142µm respectively, lower than the 300µm floc size generated by AS as primary coagulants before breakage. While OK and AS achieved their largest floc sizes after reaching the steady growth phase, it is likely that SB and KE flocs were yet to reach the steady state when the high shear was reintroduced. Furthermore, the growth rate in SB and KE was found to be slower (Fig 2); hence, their flocs needed more time to reach the steady phase before a clearer comparison can be made between the floc sizes. It is clear that the performance of SB and KE extracts as primary coagulants in terms of floc growth was very poor compared with AS flocs due to slow growth rate. Generally, floc growth rates achieved by the crude extracts as primary coagulants were very slow as seen in Fig. 2. This is because the NOM contents, especially the lipid content in the seeds has the potential to coat the surfaces of the flocs (Harold, 2001, Eman N et al., 2010). Previously,

(Xiao et al., 2011) have shown that NOM can impede the aggregation of flocs in kaolin-humic substance water sample. However, the floc growth rate was much faster in OK than in AS, demonstrating a shorter period to achieve maximum floc size, probably due to the high inter-particle bridging capability of the extract, which in real terms could result in lower cost of water treatment (Zhao et al., 2013b). In addition, when used as a primary coagulant, the OK sample exhibited good performance achieving the same floc size as that obtained by AS, (approximately 300µm). However, under these flocculation conditions, OK produced larger floc sizes due to its high protein concentration, 25% as reported by (Oyelade et al., 2003), compared to SB and KE seed with a lower protein content of 18.8% (Rao, 1996) and 13.04% (Mariod et al., 2010) respectively. Additionally, the high bridging action in OK may have been caused by the 5.09 mg of its protein in the 50 mg/l extract that was used for coagulation while in SB and KE, the amount of protein used for coagulation was 4.59 mg and 3.16mg respectively in the 50 mg/l extract.

Several studies have indicated that the main agent of coagulation in natural extracts is the presence of dimeric cationic protein with molecular mass of 6.5 and 14kDa (Ndabigengesere et al., 1995, Ghebremichael et al., 2005, Bodlund et al., 2014).

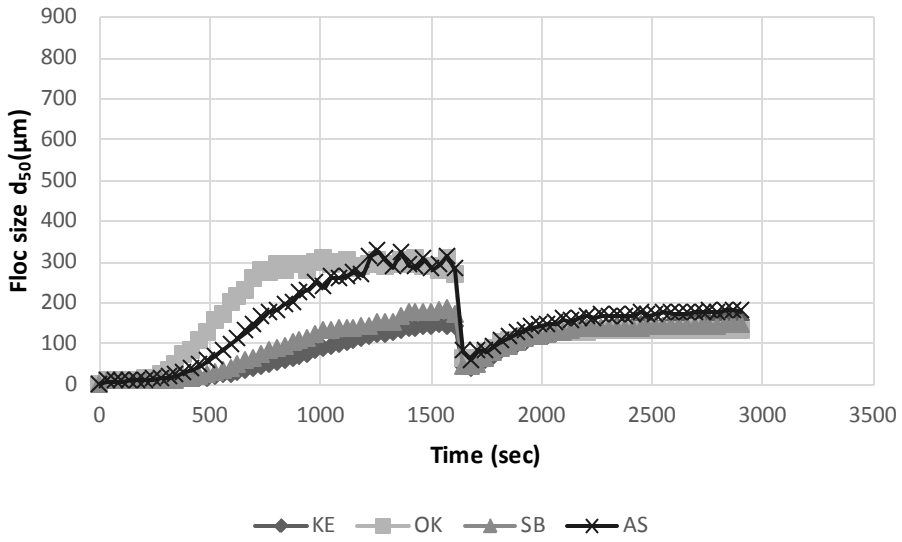
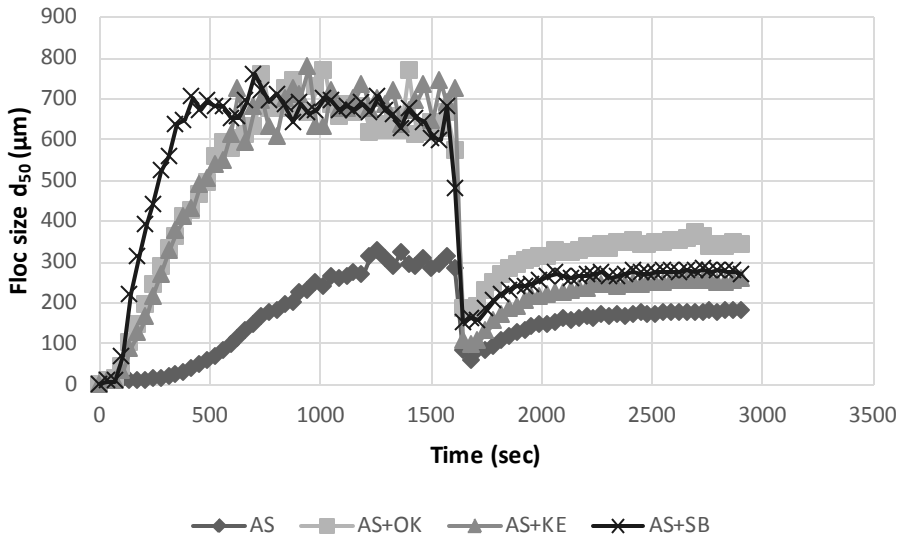


Fig 2 Floc growth, breakage and re-growth of KE, OK, SB extracts and AS used as primary coagulants

3.2 Floc growth and size of Hibiscus seed extracts as coagulant aids

The growth, breakage and re-growth factor of AS and AS+extracts flocs were evaluated as shown in Fig 3. The AS dose was 5 mg/l, as determined from preliminary jar test experiment. Similarly, 50 mg/l of each extract was employed as coagulant aids in this work. The results show that floc growth was influenced greatly by the use of crude extract samples, the effect being to increase the effective particle collision radius to give greater contact opportunity. The floc growth patterns were found to be similar for all the extracts for the duration of the experiments. It appears that floc growth of AS+OK, AS+KE and AS+SB assume a rapid growth within a few minutes of the coagulation process, although the growth was faster in AS+SB extract than in AS+KE and AS+OK samples. At steady state, when used as coagulant aids, SB, KE and OK produced floc sizes of 696µm, 701µm and 722µm respectively, but did not re-grow to their original sizes after breakage. It is believed here that the organic matter contents in the seed extracts which affected floc growth in Fig 2, was removed by employing AS as a primary coagulant. Matilainen et al. (2005) showed that AS is capable of removing up to 95% of high molecular weight NOM in water. Comparing the floc size of AS+extracts

and AS alone in Fig. 3, the d_{50} values for the AS+extract combinations were more than twice the floc size of AS used as primary coagulant (approximately 300µm). The increase in size of the AS+extract combination compared to AS alone is attributed to the double action of charge neutralisation and adsorption of AS, being further enhanced by the bridging effect of the extracts. The extracts consist of several protein molecules that contain coagulation compounds that are not limited to charge neutralisation only but exhibit absorption and bridging also (Zhao et al., 2013a), which give rise to increased floc growth. It is believed that the most important coagulation mechanism here involves charge neutralisation and patchwise adsorption of AS, which later provides adsorption sites for the extracts to form bridges with the other particles. It is clear that the addition of AS first then followed by the extracts provided the most effective flocculation process to increase floc size. This result is in agreement with work reported by Yu et al. (2009), who observed that flocs formed by charge neutralisation and bridging action are larger than flocs generated by simple charge neutralisation. The AS floc size reported in this study (approximately 300µm) is the same as that obtained by (Zhao et al., 2013b), who used *Enteromorpha* extract as a coagulant.



282

283 **Fig. 3 Floc growth, breakage and re-growth of OK, KE and SB extracts used as coagulant aids**

284

285 However, particle concentration in water has an impact on floc growth and size since the rate
286 of adsorption and bridging by natural extract increases as particle concentration increases.
287 For instance, Muyibi and Evison (1995) and Ndabigengesere et al. (1995) reported in
288 separate studies that MO extract was found to be ineffective in coagulating low turbidity
289 water. Similarly, Lee et al. (2001) used a low molecular weight polymer (10 and 50 kDa) as
290 primary coagulant and observed that the polymer was more effective in the treatment of water
291 with high turbidity. Thus the size of the floc in the work reported here may have increased
292 beyond that size if higher turbidity water had been used. However, water samples with higher
293 turbidity than the one used here were found to affect the measurement due to light
294 obscuration. Furthermore, the presence of many macro-molecules in the extracts with
295 different MW proteins and polysaccharides with long carbon chains may be responsible for
296 particle bridging. Such polymeric chains can effectively absorb colloids through absorption
297 and bridging effects as in Fig. 3, which resulted in the formation of larger flocs.

298 To examine the impact of low pH coagulation, the growth, breakage and re-growth of flocs
299 formed by OK, SB and KE seed extracts at pH 4 are presented in Fig 4. At lower pH, the

average floc sizes of SB and KE were observed to be approximately 210µm and 174µm respectively; i.e. larger than their corresponding floc sizes of 176µm and 142µm when used as primary coagulants at neutral pH. However, during the same growth period, the d_{50} floc size of OK decreased from 300µm to 240µm at pH 4. One primary cause of the decrease in floc size in OK is thought to be due to the high lipid content in the seed and partly that there may be proteins in OK seed that are sensitive to pH change and so deteriorated at low pH. The change in pH may have caused a change in the protonation pattern of the proteins especially at lower pH where protein confugiration changes. Conversely, the increase in average d_{50} floc size in SB and KE is attributed to improved coagulation efficiency between the colloids and the coagulants at low pH, because kaolin particles have been reported to be less negatively charged at pH lower than neutral (Yin, 2010).

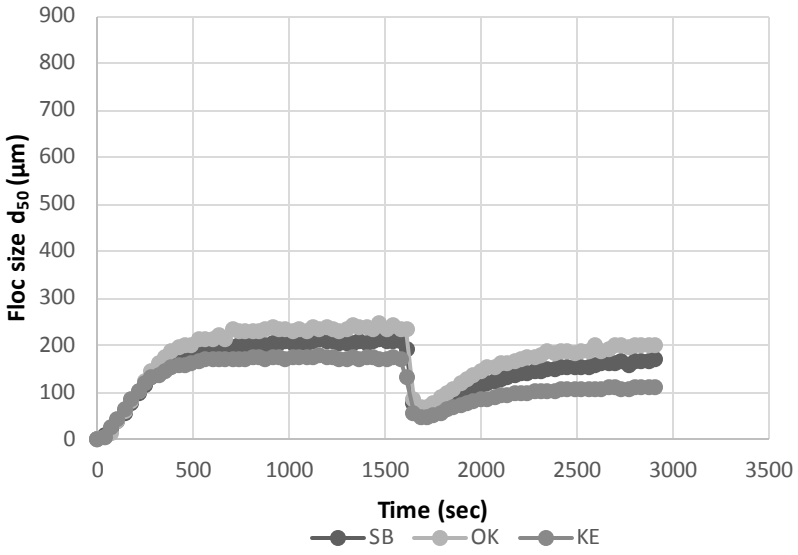


Fig 4 Floc growth, breakage and re-growth using OK, SB and KE crude extracts at pH 4.

3.3 The size of re-grown flocs of Hibiscus seed extracts

The d_{50} values for all samples were found to decrease rapidly with the re-introduction of the high shear rate at 200 rpm. The flocs began to re-grow when the slow mixing was re-introduced. Examination of Fig. 2 shows that when the extracts were used as primary

317 coagulants, whilst the size of the re-grown flocs were almost the same, (approximately
318 146μm), the breakage was most severe in OK due to its high organic matter content, because
319 flocs formed under such conditions are more fragile (Jarvis et al., 2005b). Furthermore, flocs
320 generated by KE possess a higher strength factor than flocs produced by OK or SB under the
321 same experimental condition. It is possible in this case that the ligand-binding site in KE has
322 higher affinity than those of OK and SB seeds, which provide stronger bonding with other
323 molecules. As re-growth floc sizes were similar across all the extracts, it is postulated that the
324 breakage force may have induced similar changes on the floc properties because of
325 similarities in amino acid sequence in the seeds and therefore the charge re-distribution
326 resulted in similar floc re-growth. Therefore, the use of the extract as primary coagulants is
327 not technically beneficial except that, it is affordable and easy to process to low income
328 countries.

329 Fig. 3 shows that as coagulant aids, AS+OK produced the largest regrown floc size of 350μm
330 at steady state, compared to 280μm and 274μm for AS+SB and AS+KE respectively. The
331 difference in size of the regrown flocs may have been caused by charge re-distribution after
332 breakage. As a result, each extract took a different pattern of floc re-growth which could be
333 linked to individual peptide structure (bonding sequence) of the extract. The results show no
334 significant difference between the AS+SB and AS+KE flocs at steady state before and after
335 breakage. The behaviour and response of the samples to the breaking force was similar but
336 more extensive in AS+KE followed by AS+SB whereas the amount of breakage recorded in
337 AS+OK was found to be lower. The performance of AS+OK was superior to that of AS+SB
338 and AS+KE after floc breakage, due to the high protein concentration in OK. Such behaviour
339 confirms that all the samples may possess similar protein compounds, although they may
340 differ in composition and coagulation activity.

341

342 3.4 Floc strength and recovery factors of Hibiscus seed crude extracts

343 Table 1 summarises floc strength and recovery ability, using coagulants extracted from
344 Hibiscus seeds. The results show that as coagulant aids, OK exhibited the highest strength
345 (25.5%) exceeding KE and SB (21.8% and 15.0% respectively). While floc strength factor
346 increased from 21.8% to 33.8% in KE and 15% to 25.0% in SB, the strength of the OK-
347 derived flocs deteriorated from when used as coagulant aid to when used as primary
348 coagulant. The low lipid contents in SB and KE extracts are thought to have helped in
349 improving the inter-particle bonding resulting in higher strength factors. Conversely, the
350 decrease in floc strength from (25.0% to 23.3%) in OK, is thought to be due to the presence
351 of high lipid content in the seed which can inhibit inter-particle bonding due to lack of
352 bridging action (Eman N et al., 2010, Sharp et al., 2006a). After floc breakage, a notable floc
353 recovery was seen in both SB and KE as primary coagulants (100% and 76.5%, respectively).
354 Following its low floc strength performance, OK again showed a corresponding poor floc
355 regrowth by recovering only 32.6% of its original floc size. Further evaluation of the flocs
356 after breakage when OK, SB and KE were used as coagulant aids shows that, OK recorded
357 the highest floc recovery factor of 38.6% compared with 26.6% and 23.5% recovery ability
358 recorded by SB and KE respectively. The high recovery ability in OK which coincides with
359 its high strength factor when used as coagulant aid is likely to be due to the combine effect of
360 AS plus the high protein content in the seed which improve the charge neutralisation and
361 bridging action. The work reported here observed a direct relationship between strength
362 factor and recovery factor in both OK and KE extracts. The results show that a poor floc
363 strength factor led to limited floc recovery, whereas stronger flocs exhibited a level of
364 significant floc re-growth.

365 Table 1 shows that there was very little difference between the floc strengh in OK and KE
366 extracts at low pH (29.3% in OK, 28.7% in KE and 33.3% in SB). It is clear that the acidic

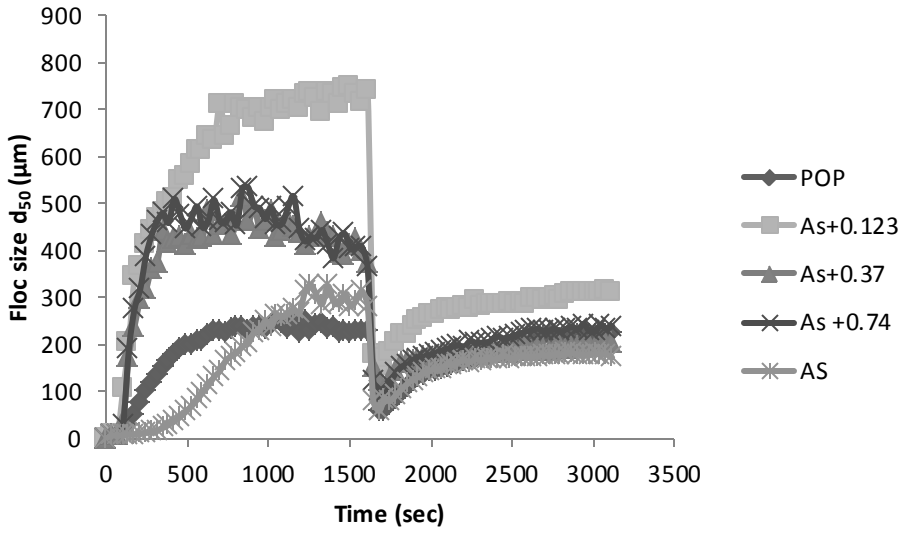
pH value played an important role in improving the strength of OK and SB, enabling adsorption sites for tighter bonding but slower floc growth, especially in OK. However, the floc strength of KE at pH 4 when used as a primary coagulant is reduced from (33.8% to 28.7%). Table 1 further shows the re-growth of flocs formed by the three samples at low pH after floc breakage, indicating a recovery ability of 46.8% for KE, 65.7% for SB and 75.7% for OK. In separate studies, Cao et al. (2010) and Sun et al. (2011) reported that flocs formed in acidic pH region were stronger and more recovarable than flocs generated in alkaline conditions. However, despite the high floc strength of 33.3% recorded at pH 4 by SB, floc re-growth was 65.7%; i.e. lower than the 100% floc recovery ability recorded when its floc strength was 25% as primary coagulant. The cause of this is likely to be due to a change in protonation pattern of SB protein at low pH which affected its binding activity during floc re-growth.

Table 1 Characteristics of floc strength and recovery factor of crude extract used as primary coagulants and as coagulant aids

	Crude coagulant		
Parameters	OK	SB	KE
Strength factor (%)			
• CE+AS @ neutral pH	25.5	15.0	21.8
• CE @ neutral pH	23.3	25.0	33.8
• CE @ pH4	29.3	33.3	28.7
Recovery factor (%)			
• CE+AS @ neutral pH	38.6	26.6	23.5
• CE @ neutral pH	32.6	100	76.5
• CE @ pH4	75.7	65.7	46.8

3.5 Floc growth and size of purified Hibiscus seed as coagulants and as coagulant aids

The performance of purified protein samples on floc growth and size as primary coagulants or as coagulant aids are presented in Figs 5, 6 and 7. In the work reported here, coagulant protein doses used in the experiments were 0.123, 0.37 and 0.74 mg/L with a pre-determined AS dosage of 5mg/L obtained from preliminary jar test results. The concentration of each of the purified proteins used in the study was found to 1.238 in POP, 1.211 in PSP and 1.092 mg/ml in PKP respectively. Fig 5 shows that the largest floc size of approximately 741µm was recorded when 0.123 mg/l of POP was added to 5 mg/l of AS, as coagulant aid. It is clear, therefore, that the purification of OK seed proteins greatly improves its performance as a coagulant aid. Further increase in POP dose from 0.123 mg/lto (0.37 and 0.74mg/L) led to a decrease in floc size, producing median d_{50} floc sizes of 490µm and 502µm respectively. This decrease in floc size is attributed to the release of excessive charged species from the combined effects of AS and POP needed for effective charge neutralisation, adsorption and bridging flocculation to occur. It is essential for successful bridging to occur using natural coagulant that sufficient particles with available unoccupied surfaces are present in order to facilitate polymer chains attachment that are adsorbed on other particles (Bolto and Gregory, 2007). In this case, subsequent addition of the coagulant proteins+AS reduced the available particle surfaces for charge neutralisation, resulting in insufficient adsorption sites for inter-particle bridging. These conditions of increasing coagulant aid dose to 0.37 and 0.74 mg/l resulted in the formation of smaller floc sizes. Interestingly, the re-growth ability of flocs formed from water coagulated with 0.37 and 0.74 mg/l of POP as coagulant aid, and when POP was used as primary coagulant, was much lower than when 0.123 mg/lwas employed as a coagulant aid. Overall, the floc sizes attained were approximately 300µm with 0.123 mg/l, 196 µm with 0.37 mg/l, and 232 µm in both 0.74 mg/l and POP despite their small pre-breakage floc sizes.



407

408 **Fig 5 Floc growth, breakage and re-growth of POP used as primary coagulant and as coagulant aids**

409 Fig 6 shows the growth, breakage and re-growth of aggregated floc formed by PSP. A faster
410 initial floc growth rate was exhibited by the PSP sample with coagulant aid doses of 0.123,
411 0.37 and 0.74 mg/l in combination with AS than when used as a primary coagulant. At steady
412 state, maximum floc sizes, d_{50} of 580µm and 519µm were achieved with 0.123 mg/l and 0.37
413 mg/l doses respectively. The flocs generated with 0.74 mg/l of PSP in conjunction with AS
414 were weaker and smaller in size, producing 491µm diameter flocs. This reduced floc size is
415 largely due to saturation of polymer bridging sites caused by the additional coagulant dose.
416 At steady state, PSP assumed a different pattern of floc growth, where the absolute deviation
417 of the median floc size about the mean value was found to be greater than in flocs generated
418 by POP. However, the pattern taken by the regrown flocs was similar for all samples
419 regardless of coagulant aid dosage and also irrespective of pre-breakage floc size. During
420 flocculation, thread-like flocs, visible to the naked eye under lamination, grow in length and
421 circumference. At the end of the measurement period, the regrown flocs reached a steady
422 phase d_{50} floc size of 243µm in all the samples, including flocs formed by PSP as primary
423 coagulant. It is noteworthy that, when used as primary coagulant, PSP produced an initial
424 median floc size, ranging between 295 and 300µm similar to the floc generated by AS as

coagulant, (approximately 300µm). However, the ionic strength of SB species was not sufficient to compress the double layer during coagulation as revealed by its surface charge potential.

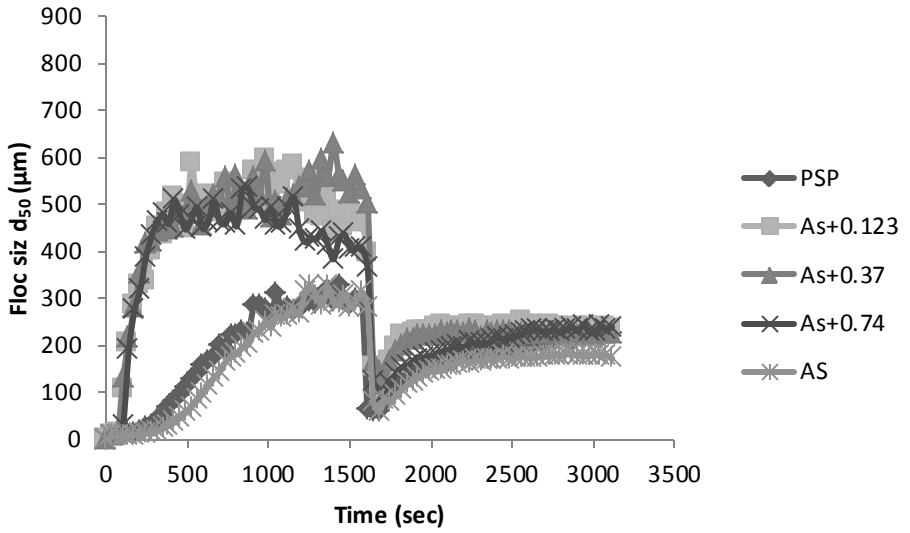


Fig 6 Floc growth, breakage and re-growth of PSP used as primary coagulant and as coagulant aids

Fig 7 shows floc growth, breakage and re-growth performance when using PKP as coagulant and as a coagulant aid. A maximum median floc size of 480µm was recorded when 0.123 mg/l of PKP was used as coagulant aid in conjunction with AS. The results show a decrease in floc size as the dosage of the coagulant aid increased, similar to the trend of floc growth shown by POP and PSP. A maximum floc size of 335µm was recorded with 0.37 mg/l of PKP and 310µm diameter floc size was generated with 0.74 mg/l dose. This again is due to insufficient adsorption sites as most of the available particle surfaces are covered with increased coagulant addition. This situation can be overcome by improving bridging flocculation conditions. La Mer (1966) postulated that optimum dosage corresponds to half of the particle surface coverage. Hence, understanding the surface charge potential in a system plays an important role in achieving enhanced floc formation during flocculation. Again, the recovery ability of floc generated by 0.37 and 0.74 mg/l of PKP as coagulant aid and PKP as

primary coagulant was found to be higher than floc recovered by 0.123 mg/l of PKP used as coagulant aid. While the d_{50} size of the regrown floc was 201µm with 0.123 mg/l dose of PKP, the 0.37 and 0.74 mg/l doses achieved post-breakage steady d_{50} sizes of 164µm and 211µm respectively. Thus, as coagulant aid, PKP exhibited the greatest floc strength and re-growth capability at a higher dose, although the initial floc size was smaller. There was a modest, yet noticeable, amount of thread-like flocs using PKP, but this was less than that observed in PSP flocs, indicating that the two seeds may have linked amino acid characteristics.

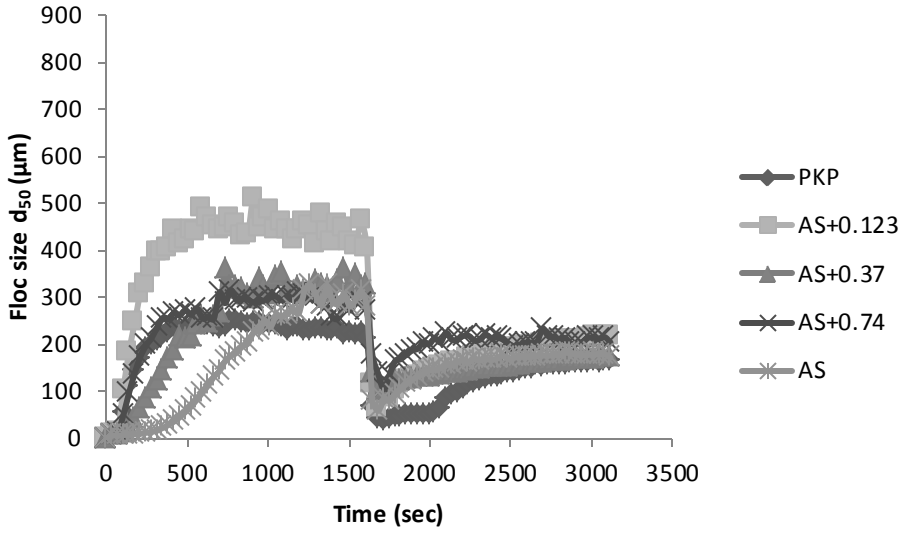


Fig 7 Floc growth, breakage and re-growth of PKP used as primary coagulant and as coagulant aids

3.6 Floc strength and recovery using purified Hibiscus seed proteins

Table 2 presents floc strength and recovery of the purified hibiscus proteins used as primary coagulants and as coagulant aids. When the purified seed proteins were used as primary coagulants, the results show that the highest strength factor of 24.3% was recorded for POP, 21.7% for PSP and 18.2 % for PKP while flocs formed by AS had a strength factor of approximately 20%. In addition, the results show that flocs formed with POP and PSP dosed as coagulant aids can resist marginally higher shear with a 0.123 mg/l dose compared to AS

459 at 5mg/l dosage under the same coagulation conditions. The high floc strength recorded by
460 the two purified samples is thought to be due to the protein contents and their sequence in the
461 seeds with a higher affinity to bind other molecules. The presence of many macromolecules
462 from natural extracts is reported elsewhere to be associated with adsorption and bridging
463 action which is believed to be the main agent for the coagulation activity (Antov et al., 2010).
464 Furthermore, the addition of the 0.123 mg/l dose as coagulant aid with AS produced larger
465 floc size with corresponding decrease in floc strength of POP and PKP, whereas the floc
466 strength of PSP remain largely unchanged . This results agrees with (Jarvis et al., 2005a) who
467 observed that the resistance of smaller flocs in turbulent flow regions is higher than that of
468 larger flocs.

469 **Table 2 Characteristics of floc strength and recovery factor of purified proteins used as primary and as**
470 **coagulant aids**

Parameters	POP	PSP	PKP	AS
Strength factor (%)				
• Primary coagulant	24.3	21.7	18.2	20
• AS + 0.123 mg/l	20.8	21.4	14.0	_
• AS + 0.37 mg/l	20.9	22.2	31.3	_
• AS + 0.74 mg/l	19.2	19.5	35.6	_
Recovery factor (%)				
• Primary coagulant	70.7	71.4	64.3	50
• AS + 0.123 mg/l	27.3	25.7	38.0	_
• AS + 0.37 mg/l	28.4	25.2	25.1	_
• As + 0.74 mg/l	36.9	31.4	59.0	_

471 Interestingly, when the coagulant dose was increased from 0.123 to 0.37 mg/l, and
472 maintaining the same shear rate, the strength factor was broadly the same for POP (20.8% to
473 20.9%) but increased slightly for PSP from 21.4% to 22.2%. In the case of PKP, the increase
474 was significantly higher, from 14.0% to 31.3%. Further increase in coagulant aid dose to 0.74
475 mg/l caused further floc strength decline in POP and PSP. It is thought that a lack of proper

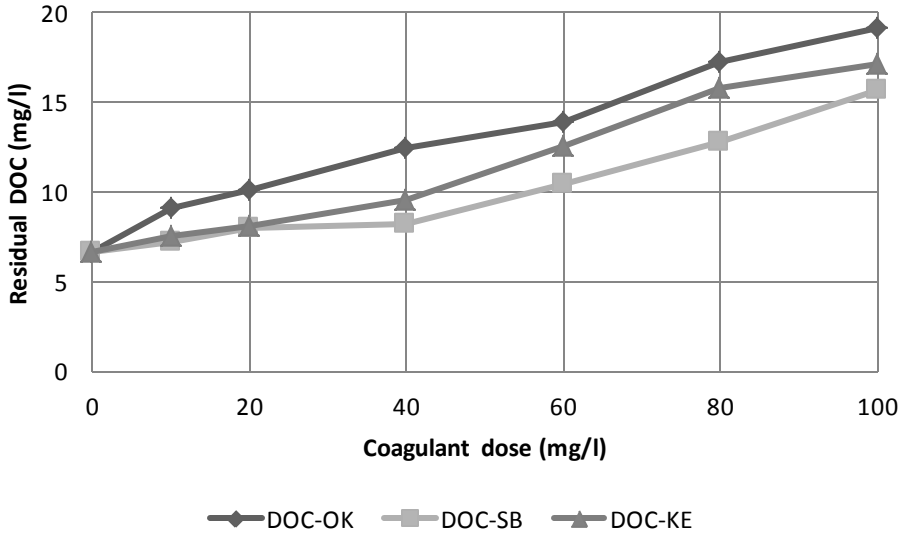
initial bonding due to polymer saturation may have been the major cause of this trend in floc strength deterioration. Under the same conditions, floc strength improved further in PKP from (31.3% to 35.6%). PKP flocs were found to behave in a similar fashion to AS in work reported by (Yu et al., 2014), who showed that an increase in alum dose during coagulation resulted in increased floc strength. The work reported here noted that an increase in PKP dosage from 0.123 to 0.74 mg/l resulted in a further increase in floc strength factor, from 18.2 to 36.0%. Although PKP has low protein content as reported earlier, flocs generated by 0.37 and 0.74 mg/l PKP were stronger than flocs formed by POP and PSP under the same dosage condition. The result demonstrated that if PKP is used as coagulant aid with AS, at a higher dose of 0.74mg/l, the improvement in floc strength was significantly higher compared with 0.123 and 0.37 mg/l doses. Further investigation revealed that floc reversibility of the purified proteins was better when the samples were used as primary coagulants, with PSP, POP and PKP re-growing to 71.4%, 70.7% and 64.3% of their original size respectively, whereas AS flocs recovered only 50% of their original size. The slight difference in floc recovery ability in PKP may be attributed to its low protein content of 10.56% as reported by (Mariod et al., 2010). However, there is a clear indication that all the seeds have some similarity in their amino acid sequence, since they are of the same species, and the redistribution of the surface charge after breakage took a broadly similar pattern. Furthermore, at a higher coagulant aid dose of 0.74 mg/l, the recovery factor improved across all the samples compared to 0.123 mg/l dose. While floc recovery ability was 59.0% in PKP and 36.9% in POP, the recovery factor was only 31.4% in PSP which was lower than the recovery ability recorded by the other samples. Again, at the 0.123 mg/l dose, floc recovery by the PKP sample was much higher than the maximum floc re-growth achieved by PSP and POP at 0.74 mg/l as coagulant aid. It is noteworthy, however, that the re-growth of PSP and PKP flocs was the same, (approximately 25% at 0.37 mg/l dose) while the regrown floc was 28.4% in POP. Nevertheless, all flocs generated by POP and PKP as coagulant aids achieved higher floc

502 recovery factors than PSP flocs under the same shear force condition, probably as a result of
503 the thread-like flocs formed by PSP being easily broken.

504

505 3.7 Effect of DOC in treated water using Hibiscus plants

506 Figure 8 shows the residual DOC concentration when water was dosed with specific Hibiscus
507 crude extract concentrations. The result reported in this work is in agreement with several
508 previous research studies (Ndabigengesere and Subba Narasiah, 1998, Okuda et al., 2001)
509 where DOC addition in final water was found to be significant. The DOC concentration
510 increased from 6.7 mg/l in raw water to 19.1, 15.7 and 17.1 mg/l when dosed with 100 mg/l
511 of OK, SB and KE, respectively. Crude extracts may contain compounds other than proteins
512 such as fats carbohydrate, fibre etc. which impacted the overall water treatment quality. The
513 organic matter from the extracts may be a surrogate for disinfection by-products (DBPs)
514 formation if chlorine is used (Liu et al., 2014).



515

516 **Fig 8 Impact of DOC additions in treated water using OK, SB and KE seed extracts.**

517

Similarly, Figure 9 shows the performance of the purified proteins in terms of DOC concentration in the clarified water. It is noteworthy that the use of POP, PSP and PSP lowered the treated water DOC in the final water. At optimum doses, DOC decreased from 6.7 mg/l to 5.0, 5.1 and 5.5 mg/l in POP, PSP and PKP treated waters. It is clear that the use of the purified proteins can reduce the impact of DBP formation in water and the purification process achieved the desired goal of obtaining the proteins in pure state. All the contaminants in the seeds that may have contributed to increasing the overall organic matter in water were removed.

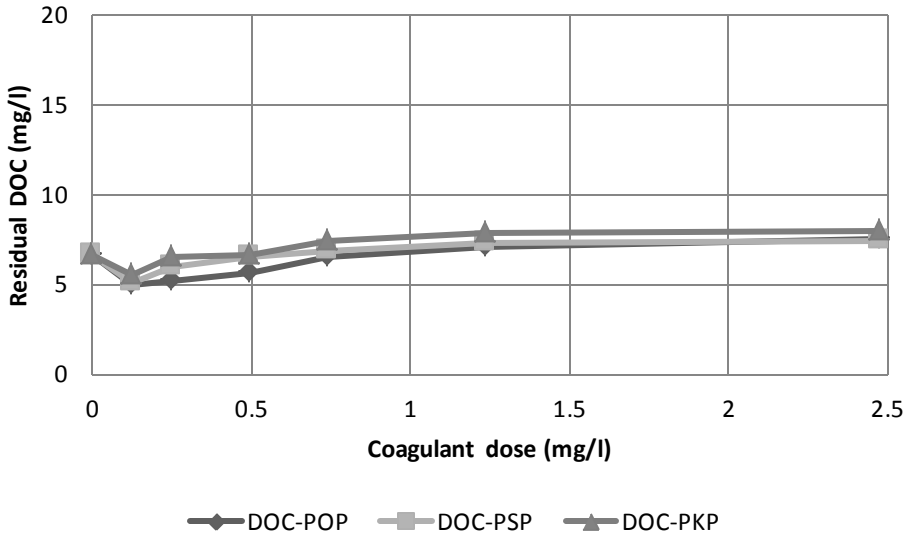


Fig 9 Impact of DOC in treated water using POP, PSP and PKP as coagulants.

4.0 Conclusions

1. When used as a coagulant aid in conjunction with AS as the primary coagulant, Hibiscus seed extracts can significantly improve floc growth and strength in water treatment. A doubling of floc size was achieved with a 0.123 mg/l dose of purified seed proteins. The floc recovery ability of POP, PSP and PKP was found to increase as coagulant aids doses increased to 0.74 mg/l, but an improved floc re-growth of

70.7%, 71.4% and 64.3% was achieved by POP, PSP and PKP when the samples were used as primary coagulants.

2. Flocs formed by PKP at a dose of 0.74 mg/l and flocs formed with 0.123 mg/l of PSP were more resistant to breakage than AS flocs, but POP flocs were strongest when it was used as a primary coagulant.
3. The application of Hibiscus seeds can help to prevent filter clogging because it generate larger flocs that can settle effectively, especially as coagulant aids. In crude form, SB and KE exhibited excellent re-growth capability (100% and 76.5%, respectively).
4. The findings support the hypothesis that the dominant flocculation mechanism for all the extracts was favoured by sorption and bridging action due to the availability of many macro-molecular proteins which are anionic.
5. The effects of Hibiscus plant seeds as coagulant aids were clearly demonstrated in this work and so a notable benefit can be derived from its application by people in low income countries, because it is non-toxic and significant cost savings can be achieved in water treatment due to the lower dose of AS requirement.

Acknowledgement

The first author gratefully acknowledges the financial support given to this research work by the Nigerian government, through the Tertiary Education Trust Fund (TETFund/AST &D/2013/2014). The authors also wish to thank Mark Carter (Civil Engineering) for helping with materials for the preparation of the coagulants and Eimear Orgill (Geography, Earth and Environmental Sciences) for her assistance with the Mastersizer for floc strength measurements.

Conflict of interest

The authors wish to declare that there are no conflicts of interest regarding the publication of this paper.

Author's contributions

The first author conducted the laboratory works and participated in the analysis of the results and writing up of the paper.

The second author participated in reviewing the experimental procedures, data analysis and writing the research paper.

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